Chapter

Immune System Modulations in Cancer Treatment: Nanoparticles in Immunotherapy

Kadriye Kızılbey, Nelisa Türkoğlu and Fatma Ceren Kırmızıtaş

Abstract

Cancer immunotherapy is based on the idea of overcoming the main problems in the traditional cancer treatments and enhancing the patient's long-term survival and quality of life. Immunotherapy methods aimed to influence the immune system, to detect and eradicate the tumors site and predict the potential results. Nowadays, nanomaterials-based immunotherapy approaches are gaining interest due to numerous advantages like their ability to target cells and tissues directly and reduce the off-target toxicity. Therefore, topics about immune system components, nanomaterials, their usage in immunotherapy and the benefits they provide will be discussed in this presented book chapter. Immunotherapy can be divided into two groups mainly; active and passive immunotherapy including their subtitles such as immune checkpoint inhibitors, adoptive immunotherapy, CAR-T therapies, vaccines, and monoclonal antibodies. Main classification and the methods will be evaluated. Furthermore, state-of-art nanocarriers based immunotherapy methods will be mentioned in detail. The terms of size, charge, material type and surface modifications of the nanoparticles will be reviewed to understand the interference of immune system and nanoparticles and their advantages/disadvantages in immunotherapy systems.

Keywords: tumor, cancer immunotherapy, vaccination, immunomodulation, antigen receptors, nanoparticles, bottom-up method, top-down method

1. Introduction

Understanding the immune system and its components may enlighten future potential treatments to generate disease progression such as cancer. For almost 30 years, by targeting the immune system by therapeutics brought a totally new point of view in the field of cancer treatment. Accordingly, besides the commonly preferred cancer treatments, the treatments developed specifically for the patient and the diseases come to the forefront. To date, immunotherapy is a method developed as an alternative to conventional cancer treatments [1, 2]. The immune system which is an awareness system based on distinguishing between "self" and "non-self" works in harmony with cells, related tissues, and organs respectively to protect

the organisms. The main goal of the immune system is to defense to battle against "enemies". There are two types of immune responses; humoral and cellular immunity. Humoral immunity is primarily mediated by B and T lymphocytes and their products. It is also characterized by a weak response and a strong immunological memory. Cellular immunity components are natural killer (NK) cells, eosinophils, macrophages, and lymphocytes (B and T cells). Cellular immunity works faster than humoral immunity via activation and proliferation of B cells and activation of antigen-presenting cells (APCs). Cellular immunity can recognize tumors immediately, but it does not provide long-term immunity. Specifically, B and T cells primarily mediate the antitumor response. The CD4+ T cells pretend as "helper cells" and excrete cytokines relying on their profile either Th1 or Th2. Humoral and cellular immunity plays a crucial role in antitumor response [3, 4].

2. Cancer

Today, non-communicable diseases are held accountable as the leading cause of death worldwide. Among these diseases, cancer is predicted as one of the most important disease in the world that causes deaths and reduces the life quality [5]. Soon, it is thought that the number of cancer patients and cancer-related deaths will increase [6]. The definition of cancer for the first time in 3000 BC was used in inscriptions called the Edwin Smith Papyrus, the part of an ancient Egyptian textbook on trauma surgery. Cancer is generally characterized by the growth of abnormal cells beyond their normal limits. Cancer disease can affect almost any part of the body and has many anatomical and molecular subtypes, each of which requires specific treatment strategies. The main factors causing cancer are as follows; ionizing radiation, ultraviolet rays, age, inadequate physical activity, smoking and alcohol consumption, nutrition and diet, chemicals, microorganisms, and genetic factors. It is known that environmental factors are much more effective in the formation of the disease than hereditary factors. The most important reason is stated as mutations that occur in genes. Most cancers are caused by a series of mutations that allow cells to divide faster, escape internal and external controls, and prevent programmed cell death. As the cells continue to divide under the influence of mutations in solid tissue such as organ, bone or muscle the resulting mass is called tumor. Solid tumors are classified as; benign (noncancerous) and malignant (cancerous). Benign tumors do not have the ability to metastasize; they can only grow where they are located. On the other hand, malignant tumors have the ability to spread to neighboring tissues and organs from where they are formed. Many types of cancer initially show no symptoms. The main symptoms observed can be given as; unexplained, and rapid weight loss, fever, malaise, pain, swelling and bleeding. However, each type of cancer has its own specific symptoms, so the treatment method of each cancer type differs. **Figure 1** shows a schematic representation of tumor cells progression.

2.1 Cancer treatment methods

Cancer is an individual disease; hence treatment methods vary from patient to patient. The method of treatment should be chosen by considering the degree and course of the disease, age and health situation of the patient. Generally, most of the patients have the combination of treatment methods. Surgical intervention, radiation therapy, chemotherapy, and hormone therapy are defined as traditional cancer treatments in the literature [2]. In recent years, immunotherapy has also been the increasingly used method in cancer treatment.

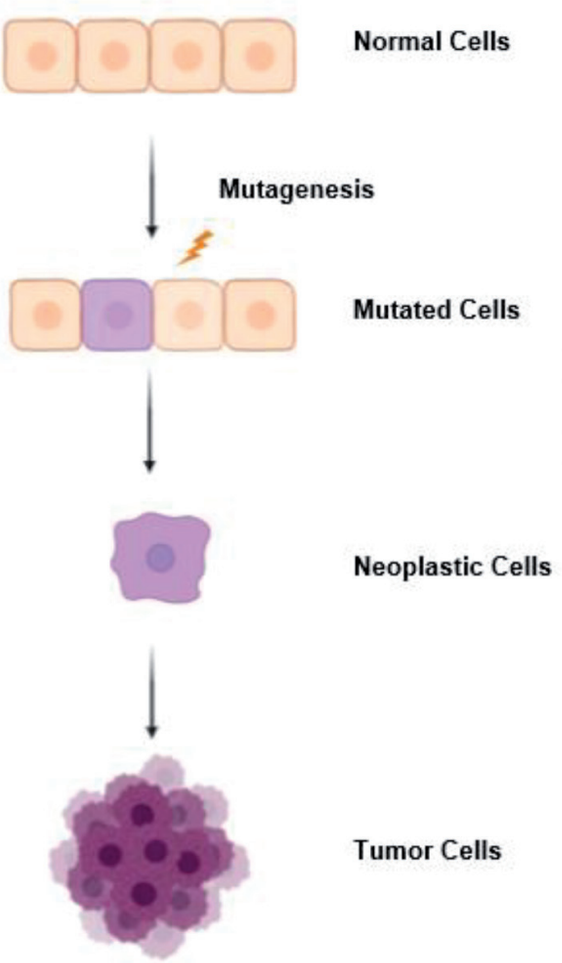

Figure 1.
Schematic representation of tumor cells progression (created with BioRender.com).

2.1.1 Surgical intervention

Surgical intervention, a local treatment method, can also be used in the combination of other treatment methods. It is applied in tumors without metastasis that only exist in one area such as solid tumors; but not effective in leukemia and cancer types that spread. Also, surgical intervention is preferred when the tumor is in an untreatable part of the body by other treatment methods such as radiation therapy or chemotherapy (cannot reach the brain). In order to remove the tumor without damaging the neighboring healthy cells, the size of the tumor can be reduced by other methods. The surgical intervention method works against cancer in three ways; eradicating the entire tumor, debulking a tumor, and palliate the disease symptoms. Only eradicating the entire tumor may cure the patients if the cancer cells are located in small area at one place. Debulking a tumor is used to reduce the tumors size while surgery is combined with other treatment methods. Palliate the symptoms, the last way in surgical intervention, is to remove the tumor to reduce the pain or pressure caused by the tumor. There are some disadvantages of surgical intervention such as the possibility of leaving microscopic residues around the tumor after surgery, the health status of the patients, and the success of the surgery [7, 8].

2.1.2 Radiation therapy

Radiation therapy or radiotherapy (RT) is based on the principle of using a fairly high dose of radiation to shrink the tumor by killing the cancer cells. There

are various types of radiotherapy depends on the general state of the patient and disease. The principle of radiation therapy is to destroy cancer cells as possible without damaging healthy cells. Because, in the late 20th century, scientist discovered that radiation therapy not only cures cancer cells but also may be the cause of cancer itself. The notable side effect, it can kill and harm healthy cells significantly. Thereby it has side effects such as hair loss, vomiting, and loss of appetite that will affect your daily life. The choice of the exact type of radiation therapy relies on several circumstances such as the type, stage, size and location of cancer, and medical history of the patients. Reducing the tumor mass by radiation therapy is helpful to decrease the pressure of tumor on the nearest healthy cells. Additionally, it is used before surgical intervention to shrink tumor mass to make it suitable for surgery and after surgery; the microscopic residues on the edge of the tumor can be removed much more easily. Also, this method of therapy is very suitable for making systemic therapy [9, 10].

2.1.3 Chemotherapy

Chemotherapy (CT), also as chemo, is the most commonly used method in cancer treatment. The aim is to kill cancer cells using chemotherapeutic agents. This method is developed in the late 20th century and combined with surgery and/or radiation therapy. Over the years, many chemotherapeutic drugs showed great impact and gained success for the treatment of many types of cancer. The aim of the treatment can be stated as reducing the size of the tumor, reducing the effects of the symptoms seen in the patient, preventing metastasis, and reducing the total number of tumor cells in the body. The drugs used in chemotherapy direct the cell to death by stopping or decelerating the cancer cell proliferation. Some of these drugs are natural and some of them are synthetic. Hair loss, vomiting, loss of appetite, fever, diarrhea and fatigue are temporary side effects of the drugs that end after the treatment [11, 12].

2.1.4 Hormone therapy

Hormones, in the classical sense, are organic compounds that are synthesized in ductless glands such as the pituitary gland, adrenal gland, thyroid gland, and parathyroid gland, which are known as endocrine organs, and act on certain target tissue that is carried by the blood. All cells communicate with each other *via* hormones. In the human body, hormones either can be small proteins (insulin, etc.) or stimulator for a cell to generate new proteins or cease making products. One possible featured outcome is cell growth and proliferation. Even though cancer cells are abnormal, they still keep the ability to react to signals of hormones. The main idea of hormone-based treatments is to deprive cancer cells of hormone signals. Otherwise, they would be stimulating to continue dividing. The main theme of the drugs that are used in this method relies on preventing the activity of hormone within the target cell or blocking the production of the related hormone. Hormone therapy is often preferred for the treatment of prostate and breast cancer. Generally, hormone therapy is combined with other treatment methods depending on the cancer type. Hormone therapy is very suitable for adjuvant and neoadjuvant therapy to reduce tumor mass. The term adjuvant therapy is about reducing the risk of cancer recurrence after major cancer treatment. Hormone therapy is also appropriate for the removal of cancer cells that spread to different parts of the body. Like all other methods, hormone therapy has common side effects. But these effects depend on the body's response to the therapy and the type of hormone therapy. Side effects are influenced by different terms such as patients' sex and type of hormone that is used. Hot flashes, weakened bones,

nausea, and fatigue are common side effects for men. Menstrual irregularities for women who are not menopausal and vaginal dryness are seen in addition to the common side effects. To date, there are several hormone based drugs based on the hormonal signals, but their principles are diverse from each other. They all attack different parts of the pathways to decelerate to the growth of cancer [13].

2.1.5 Immunotherapy

Nowadays, cancer treatment is moving from non-specific methods to specific methods. Although success is achieved in the destruction of tumors with surgery and radiotherapy, cancer may recur due to cancerous cell debris in the damaged area. Cancer immunotherapy, an individualized method, is referred to as the "fifth step" of the treatment following the traditional methods mentioned above [14]. The immunotherapy method; boost the immune system to fight against cancer, train the immune system component's to memory, attack the cancer cells, and heighten the immune response *via* biological substances. For the last decades, immunotherapy becomes a promising method to fight against cancer. Immunotherapy can be applied using either external substances or their body cells [4].

3. Historical background of cancer immunotherapy

It is common knowledge that many cases of regression of tumor growth after high fever attacks or infectious diseases have been reported throughout history from Ancient Egypt to the 18th century. However, the relationship between the immune system and cancer was noticed in the middle of the 18th century with the developing technology. In the mid-18th century, two German doctors, Busch and Fehleisen, independently reported cases of tumor regressions of patients after erysipelas infection (*Streptococcus pyogenes* infection). In the literature, the first systematic immunotherapy study for the treatment of malignant tumors was conducted in 1891 by William B. Coley, a surgical oncologist. Coley injected the heat-inactivated *Streptococcus pyogenes* and *Serratia marcescens* organisms into the patient to stimulate the patient's immune system. After the project that he initiated, Coley has seen a regression in the tumor in more than 1000 sarcoma patients who cannot undergo surgical intervention. In a very short time, humanity evaluated this mixture as a great invention, "Coley Toxins". However, the word "toxin" was an unfortunate choice; the more acceptable name for the treatment was "mixed bacteria vaccine". Although the bacteria had some side effects such as fever and malaise, it is not as toxic as chemotherapy or radiotherapy and does not destroy the immune system [15, 16]. Coley's life-long cancer immunotherapy studies that will spearhead for many scientists have started after this project. In 1900, Paul Ehrlich stated that the first findings of the treatment, which would later be called antibody-mediated passive immunotherapy, had an important place in the treatment of tumors. In 1975, George Köhler and Cesar Milstein developed hybridoma technology for monoclonal antibody production. This was followed by the first successful use of monoclonal adults in human neoplasia in 1982 and the FDA (US Food and Drug Administration) approval of muromonab-CD3 (Orthoclone OKT3) in 1986. In 1997, both the first humanized monoclonal antibody, daclizumab (Zenapax), and the first monoclonal antibody for malignancy, rituximab (Rituxan), were approved by the FDA. This was followed by the FDA approval of gemtuzumab ozogamicin (Mylotarg) in 2000, the first toxin-bound monoclonal antibody, and ibritumomab tiuxetan (Zevalin) in 2002, the first radionuclide-bound monoclonal antibody [17].

Another area that cancer immunotherapy has advanced was using the patient's body cells. In the 1960s, the tumor immune surveillance hypothesis was put forward by Burnet. Since 1995, persuasive studies on effective tumor-specific immunity have attracted great interest. In particular, many studies show the ability of dendritic cells to elicit tumor-specific T cell immunity has led to this situation. Following preclinical researches, many studies involving various types of cancer have been conducted in patients. Recent studies have also made the immunosurveillance hypothesis quite popular [18, 19]. Immunotherapy studies have increased their importance in the 21st century with the licensing of clinical studies carried out with developing technology and methods [20]. Immunotherapy was declared as "breakthrough of the year" by Science magazine in 2013 after the clinical success achieved and has become even more prominent. Also, in 2018 James Allison and Tasuku Honjo received the Nobel Prize in Physiology and Medicine for their work based on the use of the immune system to destroy cancer cells. In the past two decades, great strides have been made in cancer immunotherapy. With all these spectacular developments, the number of cancer immunotherapy studies is increasing day by day [21, 22]. There are certain categories in cancer immunotherapy applications. These are the mechanism of innate and acquired immune resistance, internal and external resistance to immunotherapy, self-neutralization of tumor cells and antigen- presenting cells, inhibition of immunity by exosome release mechanisms, the response of tumor cells to therapy. Like all other methods, cancer immunotherapy has several advantages and disadvantages. Higher precision and specificity, long-term survival rate, fewer side effects than traditional treatment methods, removing residual tumor cells and microscopic lesions that remain in the body after treatment and improving the body's immune function are the advantages of immunotherapy. Also, it can control and kill more than one tumor type and it uses the body's immune system to increase immune response. Higher treatment costs, various non-specific toxic side effects after treatment are the disadvantages of immunotherapy. There is a high selectivity for patients in treatment. When the tumor type is "immunosuppressant type" or "immune exclusion type", the effect of immunotherapy treatment is considerably weak. Additionally, in particular, the use of immune checkpoint inhibitors can have adverse consequences leading to autoimmune diseases and even death [23].

4. Classification of immunotherapy

Cancer immunotherapy is generally classified in three ways; passive, active and combination immunotherapy depending on the mechanism of the therapeutic agent and the state of the patient's immune system. Classification of passive and active cancer immunotherapy studies is shown in **Table 1**.

4.1 Passive immunotherapy

The main purpose of passive immunotherapy is to increase the current anti-tumor response by using therapeutics that can be produced under laboratory conditions. It is preferred to use the treatment in patients with weak or dysfunctional immune systems. It is designed to attack tumor cells independently by modifying the components of the immune system in the laboratory. Monoclonal antibodies and adoptive cell therapy are frequently used passive immunotherapy methods [4, 20, 24].

	Passive immunotherapy	**Active immunotherapy**
NON-SPECIFIC	Adoptive Cell Therapy	Cytokines Immune Checkpoint Inhibitors
SPECIFIC	Monoclonal Antibodies	Cancer Vaccines Oncolytic Viruses

Table 1.
Classification of immunotherapy.

4.1.1 Monoclonal antibodies

For the past 20 years, monoclonal antibodies are the most commonly used FDA approved treatment in clinical immunotherapy studies. They are large artificial proteins with high antigen specificity produced by particular B cells. Due to their antigen specificity, their capacity to bind to epitopes on the surface of the tumor cell is high [25]. So, antibodies specific to antigens of cancer cells are produced in ex vivo conditions and transferred to the patient to increase the immune response. Antibodies in these targeted therapies are guided directly to the antigen on the surface of cancer cells. Different signaling functions can be created by the interaction of monoclonal antibodies and receptors on the surface of malignant tumors. Antibodies are used in treatment can be classified as naked, conjugated, radiolabeled, chemically labeled, and bispecific monoclonal antibodies. Naked monoclonal antibodies are most commonly used in cancer immunotherapy and bind directly to the antigen without any radioactive markers or drugs. Conjugated monoclonal antibodies are used to transfer chemotherapeutic drugs or radiolabeled particles to cancer cells. Radiolabeled monoclonal antibodies are created by adding radioactive particles to naked antibodies. Chemically labeled antibodies are monoclonal antibodies with a high chemotherapeutic effect. Radioactive or chemically labeled monoclonal antibodies aim to destroy the target cell with the toxins they contain or the radiation they emit. Bispecific antibodies carry two types of antibodies in their structure and can bind to two different antigens that are receptors for these two antibodies at the same time [18, 26, 27]. The first drug including monoclonal antibodies approved by the FDA was rituximab (Rituxan, Genentech) was used in the clinic at 1997. Today, with developing technology, many new drugs have emerged for the treatment of different types of cancer [25].

4.1.2 Adoptive cell therapy

It gathered speed with the studies carried out in the 20th century about the discovery of tumor-specific antigens located not on healthy cells but just on the tumor cells. Thus the importance of adoptive T cell transfer has been understood. Adoptive cell therapy is the transfer of natural or genetically modified T cells to patients in *ex vivo* conditions instead of stimulating the immune system. The transferred cells can be autologous or allogeneic targeted to a particular antigen in the host cell. It was pointed out that the stage of an immune response in the host is skipped directly by this step. To create a targeted immune response, autologous cells can recognize tumor antigens, move towards the tumor and exit the circulation. The transfer of T cells to destroy tumor cells is carried out in two ways; the infiltrating (TIL) of tumor specific T cells from existing tumor cells and the use of genetically modified T cells to specifically identify tumor cells. In both methods, the T cell is processed *ex vivo* and then transferred back to the patient [28]. The first successful cellular therapy in history was performed on an advanced melanoma patient with autologous TIL. The specific T cell receptor (TCR) is obtained by genetically modifying T cells. T cells

and tumor-specific antigens are matched with HLA recognition by TCR technology. A minimal cytotoxic effect occurs by this natural pairing. TCRs also have disadvantages such as the low expression on the surface and short lifespan of T cells *in vivo*. Although the first studies ended up with disappointment, today, the other genetically modified T cell is chimeric antigen receptors, CAR. Many studies are conducted around the world on CAR-T technology and it is believed that positive results will be achieved in the near future [29, 30].

4.2 Active immunotherapy

Active immunotherapy aims to destroy cancer cells by stimulating the immune system by vaccination, immunomodulation, or targeting specific antigen receptors. The method is carried out employing cancer vaccines, oncolytic viruses, immune checkpoint inhibitors, and cytokines [20].

4.2.1 Cancer vaccines

The purpose of vaccination is to create an immune response to detect and destroy cancer cells. Cancer vaccines, containing whole, part, or purified antigens of tumor cells, can be peptide-based, immune cell or dendritic cell-based, or tumor cell-based. After the tumor cells are removed from the body, the patient is vaccinated and an immune response is created against the tumor cells that may remain in the body. Variable antigen expression, low immune response, diminishing the immune response in the tumor microenvironment and a decrease in activity over time are the restrictions of the cancer vaccine applications [4, 25].

- **Peptide-based vaccines** are designed to create an immune response against tumor antigens that interact with HLA molecules on the surface of tumor cells. Their toxic effects on healthy cells are low due to their antigen-specific design, but tumor antigen peptides and the patient's HLA type should be well characterized [31].

- **Immune or dendritic cell-based vaccines** consist of the use of tumor-associated antigens or autologous tumor cells and dendritic cells (DC) obtained from monocyte cells in early-stage cancer vaccines. In 2010, the drug called Sipuleucel-T (Provenge, Dendreon Corp.) is the first DC-based cancer vaccine was approved by the FDA for the treatment of prostate cancer. DC-based vaccines today use innovative *in vitro* culturing techniques enriched with cytokines, enhancing immunogenicity and improving DC function. DC-based cancer vaccines can be designed differently for both *ex vivo* and *in vivo* applications for various cancer types [4].

- **Tumor cell-based cancer vaccines** use the entire tumor cell to create an immune response. Unlike peptide-based vaccines, tumor cells are not specific to antigens on their surface, but the range of epitopes to which they can bind is wider. These vaccines can be prepared using the patient's cells (autologous) or using another patient's tumor cells (allogeneic). Tumor cell-based vaccines such as M-Vax (AVAX Technologies) can be used in the treatment of many different types of cancer in clinical studies [25].

4.2.2 Oncolytic viruses

These are called genetically altered viruses that can naturally penetrate only cancer cells and kill them. Talimogene laherparepvec (T-Vec) is the first oncolytic

virus-based drug, approved by the FDA, that the protection mechanisms developed against viral infections are impaired in most cancer cells. By taking advantage of this degradation, viruses can reproduce more intensely in cancer cells than healthy cells. Recently, replication specific to cancer cells was obtained and a reovirus variant called Reolysin (exhibiting oncolytic behavior in cells with activated Ras signaling pathway) has been developed. In 1991, positive results were gained in the treatment of brain cancer with a mutation in a genetically modified type 1 herpes simplex virus [32].

4.2.3 Immune checkpoint inhibitors

Several inhibitory receptors and ligands expressed on T cells, antigen-presenting cells, and tumor cells have recently been important elements of immunosuppression in the tumor microenvironment. Because of their biological role as regulators of T cell activation, these receptor/ligand pairs have been termed "immune checkpoints". Immune checkpoints are cell membrane proteins involved in the regulation of the immune response. Multiple controls or "checkpoints" are present or activated to ensure that the immune-inflammatory response is not continuously activated after tumor antigens have generated a response. Immune checkpoints are signals that can halt an existing immune response. The over -expression of these signals by tumor cells affects tumor cell-specific T-cell immunity in the cancer microenvironment. The aim of treatments involving inhibition of the immune checkpoint is to use and strengthen the immune system by disrupting the negative immune system. In 2011, the drug called Ipilimumab was used in clinical use for melanoma patients by using immune control point drugs. As of March 2019, 7 immunotherapy drugs based on checkpoints are used in clinical practice. Monoclonal antibodies that bind to immune checkpoints bind with cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand (PDL-1) [33].

- **PD-1/PDL-1**, under normal circumstances, PD-1 has two ligands; PD-L1 and PD-L2. Blocking the interaction between PD-1 and PDL-1 with antibodies enhances the immune response against cancer cells, "releases the brakes" in the immune system and allows for the attack of tumor cells that express PDL-1. Nivolumab and Pembrolizumab are the first two drugs approved by the FDA in 2014 [34].
- **CTLA-4** inhibition increases the activation of cytotoxic T cells. Thus, immune blockade due to Treg cells is inhibited and antitumor activation is observed. Ipilimumab is the first drug approved by the FDA for CTLA-4 treatment in 2011 [35].

4.2.4 Cytokines

They are the main regulators of innate and adaptive immune systems that allow cells of the immune system to communicate in paracrine and autocrine systems over short distances. Unlike other therapeutic agents, these molecules directly stimulate immune cells, for example Interleukin-21 (IL-21) can act as agents involved in active immunotherapy [36]. The use of cytokines in cancer immunotherapy showed tumor regression, prevention of metastasis formation, improvement of immunological memory and decrease in risk of disease recurrence with increased survival. The use of cytokine (IL-2, GM-CSF, IFN-α) -based biological therapy in combination with conventional therapies is under clinical development [37]. In 1986, IFN-α became the first FDA approved cytokine for the treatment of leukemia.

Subsequently, IL-2 was approved by the FDA in 1992 for metastatic kidney cancer and 1998 for advanced melanoma treatment [36].

4.3 Combinational immunotherapy

Combinational immunotherapy refers the use of a different anticancer agent for treatments of cancer. Conjugation of IL-2 and HER-2 monoclonal antibody proved to be a very forceful combination in immunotherapy. Lately, PD-1 and CTLA-4 conjugation has been examined. The results revealed that the combined system was safe and had no significant toxic effect [38].

5. Nanoparticles in cancer immunotherapy

Nanoparticle-based biomaterials have a critical role in cancer immunotherapy compared with conventional drugs [39]. Immunotherapy often targets tumor cells, immune and stromal cells in the tumor microenvironment [40]. Additionally, side reactions occurring due to the interactions between nanoparticles (NPs) and cells can be adjusted by modifications of nanoparticles [41]. Nanoparticle-based drug delivery systems can improve the solubility, *in vivo* stability, and pharmacokinetic profile. Also, they protect drugs from premature release and degradation in the living system. These systems can be designed according to the microenvironment of the target such as pH, redox potential or enzymes, and external dynamics such as light, electrical and magnetic fields. Targeted delivery with NPs can also reduce toxicity and immune-related side effects [2]. The size and the shape of the NP are very effective in therapeutic efficacy by changing its pharmacokinetics, transportation, and cellular uptake [42]. Recent advances in nanoparticle formulations have generated a wide range of other shapes like rods, prisms, cubes, stars, and discs out of spherical. It is considered as non-spherical particles have higher blood circulation periods, prolonged margination effects, and higher penetration capacities within solid tissues and tumors [43]. The charge of NP has great priority in the transition of it into cells. Besides, NP-ligand coupling conditions and the elasticity of NP upgrade transportation and accumulation of NP in the living system [44, 45]. Generally, it is well known that cationic NPs create a higher immune response than neutral or anionic NPs [43]. The size, shape, elasticity, optical, magnetic, and electrical properties of nanoparticles can be modified to increase the usage of NPs in cancer therapy as a carrier [2, 41, 46]. High specificity, efficacy, diagnosing, imaging, and therapeutic properties make NPs candidates in immunotherapy for effective cancer treatment. Liposomes, micelles, polymeric, metallic, and inorganic NPs have a wide range of usage in cancer immunotherapy [44].

5.1 Classification of nanoparticles

The nanoparticles are generally categorized into tree class as organic, inorganic, and carbon-based. Dendrimers, micelles, and liposomes are the most widely known organic nanoparticles. These biodegradable, non-toxic, and capsule-shaped nanoparticles appear to be an ideal choice for drug delivery due to their sensitivity to thermal and electromagnetic radiation. Inorganic nanoparticles, metal, and metal oxide-based NPs do not contain carbon in their structure. Aluminum, cadmium, cobalt, copper, gold, iron, lead, silver, and zinc can be used to fabricate metallic NPs in 10 to 100 nm size range. Carbon-based nanoparticles, fullerenes, graphene, carbon nanotubes (CNT), and carbon nanofibers, are build up from carbon in nanosize [47].

5.2 Preparation methods of nanoparticles

It can be viewed two different ways to synthesize nanoparticles; bottom-up and top-down methods (**Table 2**). These techniques also can be divided as chemical and physical methods. Although both methods have positive and negative features, the chemical one has more disadvantages due to the wet reaction steps it has [48].

5.2.1 Bottom-up method

It is also known as a constructive method like building-up of material from an atom. Sol–gel, spinning, chemical vapor deposition (CVD), pyrolysis, and biosynthesis are the foremost methods in this technique. Nanoparticles, nanoshells, and nanotubes with narrow size distribution can be synthesized by this approach. Besides, in this method deposition parameters can be controlled. However large scale production is difficult and chemical purification is needed.

- **Sol–gel method:** It is a simple, wet chemical process based on hydrolysis and polycondensation reactions [49]. This process indicates the chemical transformation of a system from a "sol" phase, a colloidal solution of solids suspended in a liquid phase, into a "gel" phase, a solid macromolecule submerged in a solvent [50]. The chemical and physical properties of the materials as high surface area and the stability can be obtained by the method via modifying experimental conditions. Metal oxide and chloride precursors are used in sol–gel process, and then a liquid and a solid phase separation occur after removing precursors either by shaking, stirring, or sonication. Nanoparticles are acquired in this phase separation by sedimentation, filtration, or centrifugation [47].

- **Spinning disc processing (SDP):** The method consists of a rotating disc inside a reactor generally filled with nitrogen or other inert gases to remove oxygen inside and avoid chemical reactions. The purpose of spinning is to merge atoms or molecules. The parameters of this process such as the liquid flow rate, disc rotation speed, liquid/precursor ratio, location of feed, and disc surface may vary for different systems and determine the characteristics of NPs [51].

Top-down approach		**Bottom-up approach**	
Physical processing methods		Physical and chemical processing methods	
		Physical techniques	
Mechanical methods:	Cutting, etching, grinding	Physical Vapor Deposition (PVD):	Evaporation (thermal, e-beam)
	Ball milling		Sputtering
Lithographic techniques:	Photo Lithography		Plasma Arching
	Electron Beam Lithography		Laser Ablation
		Chemical techniques	
		Chemical Vapor Deposition (CVD):	Plasma enhanced CVD (PECVD)
		Self Assembling	Electronic deposition, sol-gel method, emulsion, pyrolysis

Table 2.
Techniques in Top-Down and Bottom-Up approaches.

- **Chemical vapor deposition (CVD):** It is the deposition technique of thin films of gaseous reactants onto a substrate. Gaseous reactants can be elemental and compound semiconductors, metal alloys, and amorphous or crystalline compounds. In the CVD process, a volatile material (chemically reactive) is coming together with other gases to produce a nonvolatile solid material that deposit at the atomic level on a suitable substrate. This is a well-organized process that some kind of reactors should be used depending on the type of precursors, deposition conditions, and the forms of the energy introduced to the system to stimulate the planned chemical reaction. Metal–organic, plasma-enhanced, low-pressure, laser-assisted, and aerosol-assisted CVDs are the most accepted methods [52]. The deposition is carried out in a reaction chamber at the temperature suitable for the reaction, the substrate is heated and the chemical reaction occurs when the heated substrate contact with the combined gas. The substrate temperature is an important parameter for this method to gain pure, uniform, hard, and strong nanoparticles [47, 53].

- **Spray pyrolysis**: It is a method often used in industry for large scale production of NPs. Generally, nanometals and metal oxides are produced by this simple, reproducible, size controllable and low-cost method [54]. This process consists of a precursor with flame where the precursor solution is sprayed or injected using a nanoporous nebulizer onto the hot substrate into the furnace at high pressure to form a droplet. The precursor can be either liquid or vapor. After evaporation, the precursor decomposes to recover nanoparticles or films on the substrate. Some of the furnaces have laser or plasma to produce high temperature to facilitate evaporation [55].

- **Biosynthesis:** It is an alternative to conventional physical and chemical nanoparticle synthesizing methods. Plants are preferred in this green and environmentally friendly cost-effective technique to prepare non-toxic and biodegradable nanoparticles [56]. In this method, several microorganisms as bacteria, fungus, and yeasts, etc. are used along with the precursors to produce nanoparticle for bioreduction and capping purposes. The biosynthesized nanoparticles have unique and enhanced properties that find a wide range of applications in drug delivery systems [57].

5.2.2 Top-down method

This method is also known as a destructive method due to the reduction of bulk material to nanometric scale particles. Contrary to bottom-up, large-scale production is possible and chemical purification is unnecessary in the top-down method. Broad size distribution (10–1000 nm), varied particle shapes, control over deposition parameters and reaction costs are disadvantages of this method. There are many techniques in this method, but mechanical milling, nanolithography, laser ablation and sputtering are among the most frequently used ones.

- **Mechanical milling**: This process has been used for a long time in mineral, ceramic processing, and powder metallurgy industries. The aim of mechanical milling of materials consists of minimizing particle size, blending, changing particle shapes, and synthesizing nanoparticles in a high energy mill with a convenient medium. At nanoparticle synthesis elements are granulated in an inert atmosphere. Mechanical milling is an economical method for nanosize production of large quantities [58]. The dynamics of mechanical milling vary according to energy transfer to the material from the balls [59]. Type of mill, the powder

supplied to drive the milling chamber, milling speed, size and size distribution of the balls, dry or wet milling, the temperature of milling and the duration of milling are the factors that affect the energy transfer. Also, deformations, fractures, and the type of welding cause variations in particle shape and size [58].

- **Nanolithography**: This is the fabrication of molecules in a nanometric size range of 1 to 100 nm. Lithography is a combination of deposition and etching to have high-resolution topography. There are two main methods called as masked and maskless lithography. These 2 methods contain many techniques inside. While a mask or a mold is needed in masked lithography to fabricate patterns, maskless lithography produces unstable patterns without the use of mask. Photolithography, soft lithography, and nanoimprint lithography are the main techniques in masked lithography. Maskless lithography consists of electron beam lithography, ion beam lithography, and scanning probe lithography [60].

  The process is about printing material in a required shape or structure on a light- sensitive material. The main advantage of nanolithography is to make several copies with the desired shape and size from a single nanoparticle. On the other hand, the necessity of some equipment and their costs are the disadvantages of nanolithography [61].

- **Laser ablation (LA):** The laser irradiates the surface of the sample with a changeable wavelength of the laser and the refractive index of the solid or liquid target material in this complex PVD process. The laser removes electrons from the target material in a high electric field and those scattered electrons meet with the atoms of the bulk sample, where the energy transfer occurs. This leads to the heating of the surface and vaporization. The material is converted to a plasma state at high laser flux. There are some different applications in this method such as welding, cladding, cutting, cleaning, and generation of nanoparticles. During applications environmental conditions such as vacuum, air, gas and liquid can be changed. Pulsed-laser ablation types of solid target materials have great potential in the fields of laser-material microprocessing, nanotechnology and device fabrication. Besides, Laser Ablation Synthesis in Solution (LASiS) is a common and reliable top-down method that provides an alternative solution to the conventional chemical synthesis of metal-based nanoparticles. Also, organic solvents and water can be used in LASiS for NP synthesis and the method can be called as a 'green' process [62, 63].

- **Sputtering:** The principle of this physical process is to use the energy of plasma on the surface of a material, to arrange the atoms of the material and deposit them on the substrate with energetic ions. After the bombardment with ions, the ejection of atoms from the target occurs and then they deposit onto a substrate in the vacuum sputtering chamber. This high vacuum-based coating technique is included in the group of PVD processes. The shape, size, and composition of the nanoparticles vary with the layer thickness, temperature, and annealing time and substrate type [64].

6. Conclusion

The application of polymeric NPs in cancer therapy has been studied for decades. Poly(lactic-co-glycolic acid) (PLGA), chitosan, and polyethylene glycol

(PEG) are the most common, FDA-approved polymeric carriers for drug and bio-agent delivery. PLGA and chitosan contain hydrophobic domains which also capable of activating immune cells by their adjuvant character. In general, PLGA-based NPs for cancer immunotherapy is based on targeting dendritic cells. Micelles and liposomes are also convenient for the delivery of therapeutics and antigens. Recently, immunomodulatory nanoliposomes with 1oo nm size were designed to deliver cancer antigens. The researches continued until today has indicated the importance of NPs in cancer immunotherapy. The antigen-NP conjugated systems help to introduce the immune-therapeutic agent to antigen-presenting cells efficiently. A high immune effect occurs with the presence of immunotherapeutic agent-loaded nano delivery systems in comparison to free immunotherapeutic agents. Prolongation, antigenicity, adjuvant selection, and inflammation are the most critical parameters for designing and engineering NPs.

On the other hand, there are still some issues to be solved in cancer immunotherapy. In some cases, insufficient information about cancer cells causes drugs not to present the expected effect. Scientists are unable to have precise information about the behavior of nanoparticles in the living system. In addition to these, there are difficulties in adjusting the toxicity, characterization, and monitoring behavior of nanomaterials in biochemical pathways. Moreover, failure to comply with the rules in drug use in such practices makes the work of the researchers even more difficult.

Besides, nanotechnology is promising for oncological applications for precise diagnoses and struggles with cancer cells. In light of the information mentioned in the literature, it is seen that interdisciplinary approaches and researches about the design and development of nanoparticle-based cancer immunotherapy are promising. Nanotechnology-based studies enable a therapeutic efficacy with a low dose of therapeutics, avoid cytotoxicity, and not to destroy the healthy cells of the patient. The quality and duration of cancer patients' lives can be improved by developing new methodologies in cancer immunotherapy based on nanoparticles.

Author details

Kadriye Kızılbey[1*], Nelisa Türkoğlu[2] and Fatma Ceren Kırmızıtaş[2]

1 İstanbul Yeni Yüzyıl University, Biomedical Engineering Department, İstanbul, Turkey

2 Yıldız Technical University, Department of Molecular Biology and Genetic, İstanbul, Turkey

*Address all correspondence to: kadriyekizilbey@gmail.com

References

[1] P. Huang *et al.*, "Nano-, micro-, and macroscale drug delivery systems for cancer immunotherapy," *Acta Biomaterialia*. 2019, doi: 10.1016/j.actbio.2018.12.028.

[2] J. Nam, S. Son, K. S. Park, W. Zou, L. D. Shea, and J. J. Moon, "Cancer nanomedicine for combination cancer immunotherapy," *Nature Reviews Materials*. 2019, doi: 10.1038/s41578-019-0108-1.

[3] H. Zhang and J. Chen, "Current status and future directions of cancer immunotherapy," *Journal of Cancer*. 2018, doi: 10.7150/jca.24577.

[4] R. Kokate, "A Systematic Overview of Cancer Immunotherapy: An Emerging Therapy," *Pharm. Pharmacol. Int. J.*, 2017, doi: 10.15406/ppij.2017.05.00112.

[5] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA. Cancer J. Clin.*, 2018, doi: 10.3322/caac.21492.

[6] V. K. Chaturvedi, A. Singh, V. K. Singh, and M. P. Singh, "Cancer Nanotechnology: A New Revolution for Cancer Diagnosis and Therapy," *Curr. Drug Metab.*, 2018, doi: 10.2174/1389200219666180918111528.

[7] A. Sudhakar, "History of Cancer, Ancient and Modern Treatment Methods," *J. Cancer Sci. Ther.*, 2009, doi: 10.4172/1948-5956.100000e2.

[8] O. Baykara, "Kanser Tedavisinde Güncel Yaklaşımlar," *Balıkesır Sağlık Bilim. Derg.*, 2017, doi: 10.5505/bsbd.2016.93823.

[9] J. R. Robbins, J. M. Longo, and M. Straza, "Radiation therapy," in *Cancer Regional Therapy: HAI, HIPEC, HILP, ILI, PIPAC and Beyond*, 2019.

[10] K. E. Burton, "Radiotherapy," in *Management of Adult Glioma in Nursing Practice*, 2019.

[11] V. T. DeVita and E. Chu, "A history of cancer chemotherapy," *Cancer Research*. 2008, doi: 10.1158/0008-5472.CAN-07-6611.

[12] B. A. Chabner and T. G. Roberts, "Chemotherapy and the war on cancer," *Nature Reviews Cancer*. 2005, doi: 10.1038/nrc1529.

[13] A. Nicolini, *G. Rossi*, P. Ferrari, R. Morganti, and A. Carpi, "A new immunotherapy schedule in addition to first-line hormone therapy for metastatic breast cancer patients in a state of clinical benefit during hormone therapy," *J. Mol. Med.*, 2020, doi: 10.1007/s00109-020-01881-3.

[14] N. Zaidi and E. M. Jaffee, "Immunotherapy transforms cancer treatment," *Journal of Clinical Investigation*. 2019, doi: 10.1172/JCI126046.

[15] E. F. McCarthy, "The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas.," *Iowa Orthop. J.*, 2006.

[16] S. J. Oiseth and M. S. Aziz, "Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead," *J. Cancer Metastasis Treat.*, 2017, doi: 10.20517/2394-4722.2017.41.

[17] T. A. Waldmann, "Immunotherapy: Past, present and future," *Nature Medicine*. 2003, doi: 10.1038/nm0303-269.

[18] M. Barbaros, Burak; Dikmen, "Kanser İmmünoterapisi," *Erciyes Üniversitesi Fen Bilim. Enst. Derg.*, 2015.

[19] C. R. Parish, "Cancer immunotherapy: The past, the present and the future," *Immunology and Cell Biology*.

2003, doi: 10.1046/j.0818-9641.2003.01151.x.

[20] N. E. Papaioannou, O. V. Beniata, P. Vitsos, O. Tsitsilonis, and P. Samara, "Harnessing the immune system to improve cancer therapy," *Annals of Translational Medicine*. 2016, doi: 10.21037/atm.2016.04.01.

[21] M. Sambi, L. Bagheri, and M. R. Szewczuk, "Current challenges in cancer immunotherapy: Multimodal approaches to improve efficacy and patient response rates," *Journal of Oncology*. 2019, doi: 10.1155/2019/4508794.

[22] M. J. Smyth and M. W. L. Teng, "2018 Nobel Prize in physiology or medicine," *Clinical and Translational Immunology*. 2018, doi: 10.1002/cti2.1041.

[23] S. Tan, D. Li, and X. Zhu, "Cancer immunotherapy: Pros, cons and beyond," *Biomedicine and Pharmacotherapy*. 2020, doi: 10.1016/j.biopha.2020.109821.

[24] D. V. Yuzhakova, M. V. Shirmanova, T. F. Sergeeva, E. V. Zagaynova, and K. A. Lukyanov, "Immunotherapy of cancer (Review)," *Sovremennye Tehnologii v Medicine*. 2016, doi: 10.17691/stm2016.8.1.23.

[25] C. Lee Ventola, "Cancer immunotherapy, part 1: Current strategies and agents," *P T*, 2017.

[26] A. A. Özlük, M. G. Oytun, and D. Günenç, "Kanser immünoterapisi," *İstanbul Bilim Üniversitesi Florence Nightingale Transplant. Derg.*, 2017, doi: 10.5606/FNG.TRANSPLANTASYON.2017.004.

[27] I. Kimiz-Gebologlu, S. Gulce-Iz, and C. Biray-Avci, "Monoclonal antibodies in cancer immunotherapy," *Molecular Biology Reports*. 2018, doi: 10.1007/s11033-018-4427-x.

[28] Ö. Met, K. M. Jensen, C. A. Chamberlain, M. Donia, and I. M. Svane, "Principles of adoptive T cell therapy in cancer," *Seminars in Immunopathology*. 2019, doi: 10.1007/s00281-018-0703-z.

[29] P. Ataca and O. Arslan, "Chimeric Antigen Receptor T Cell (Car T Cell) Therapy In Hematology," *Turkish J. Hematol.*, 2015, doi: 10.4274/tjh.2015.0049.

[30] E. Wrona and P. Potemski, "A novel immunotherapy — The history of CAR T-cell therapy," *Oncology in Clinical Practice*. 2019, doi: 10.5603/OCP.2019.0016.

[31] K. Kaur and G. L. Khatik, "Cancer Immunotherapy: An Effective Tool in Cancer Control and Treatment," *Curr. Cancer Ther. Rev.*, vol. 16, no. 1, pp. 62-69, 2020.

[32] G. Marelli, A. Howells, N. R. Lemoine, and Y. Wang, "Oncolytic viral therapy and the immune system: A double-edged sword against cancer," *Frontiers in Immunology*. 2018, doi: 10.3389/fimmu.2018.00866.

[33] S. Khan and D. E. Gerber, "Autoimmunity, checkpoint inhibitor therapy and immune-related adverse events: A review," *Seminars in Cancer Biology*. 2019, doi: 10.1016/j.semcancer.2019.06.012.

[34] K. KAHVECİ and M. Türkoğlu, "İmmün Kontrol Noktası İnhibitörleri Ctla-4 ve Pd-1/Pd-l1'in İmmünoterapideki Yeri," *Mehmet Akif Ersoy Üniversitesi Fen Bilim. Enstitüsü Derg.*, vol. 10, no. 2, pp. 210-218.

[35] J. A. Seidel, A. Otsuka, and K. Kabashima, "Anti-PD-1 and anti-CTLA-4 therapies in cancer: Mechanisms of action, efficacy, and limitations," *Frontiers in Oncology*. 2018, doi: 10.3389/fonc.2018.00086.

[36] T. A. Waldmann, "Cytokines in cancer immunotherapy," *Cold Spring*

Harb. Perspect. Biol., 2018, doi: 10.1101/cshperspect.a028472.

[37] S. Lee and K. Margolin, "Cytokines in cancer immunotherapy," *Cancers*. 2011, doi: 10.3390/cancers3043856.

[38] J. Ciccolini, D. Barbolosi, N. André, S. Benzekry, and F. Barlesi, "Combinatorial immunotherapy strategies: most gods throw dice, but fate plays chess," *Annals of Oncology*. 2019, doi: 10.1093/annonc/mdz297.

[39] C. Wang, Y. Ye, Q. Hu, A. Bellotti, and Z. Gu, "Tailoring Biomaterials for Cancer Immunotherapy: Emerging Trends and Future Outlook," *Advanced Materials*. 2017, doi: 10.1002/adma.201606036.

[40] S. K. Rajendrakumar *et al.*, "Self-assembled, adjuvant/antigen-based nanovaccine mediates anti-tumor immune response against melanoma tumor," *Polymers (Basel).*, vol. 10, no. 10, p. 1063, 2018.

[41] Y. Yu and J. Cui, "Present and future of cancer immunotherapy: A tumor microenvironmental perspective," *Oncology Letters*. 2018, doi: 10.3892/ol.2018.9219.

[42] R. Agarwal, V. Singh, P. Jurney, L. Shi, S. V. Sreenivasan, and K. Roy, "Mammalian cells preferentially internalize hydrogel nanodiscs over nanorods and use shape-specific uptake mechanisms," *Proc. Natl. Acad. Sci. U. S. A.*, 2013, doi: 10.1073/pnas.1305000110.

[43] W. Park, Y.-J. Heo, and D. K. Han, "New opportunities for nanoparticles in cancer immunotherapy," *Biomater. Res.*, 2018, doi: 10.1186/s40824-018-0133-y.

[44] S. P. Surendran, M. J. Moon, R. Park, and Y. Y. Jeong, "Bioactive nanoparticles for cancer immunotherapy," *International Journal of Molecular Sciences*. 2018, doi: 10.3390/ijms19123877.

[45] V. Manolova, A. Flace, M. Bauer, K. Schwarz, P. Saudan, and M. F. Bachmann, "Nanoparticles target distinct dendritic cell populations according to their size," *Eur. J. Immunol.*, 2008, doi: 10.1002/eji.200737984.

[46] B. D. Chithrani, A. A. Ghazani, and W. C. W. Chan, "Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells," *Nano Lett.*, 2006, doi: 10.1021/nl052396o.

[47] A. M. Ealias and M. P. Saravanakumar, "A review on the classification, characterisation, synthesis of nanoparticles and their application," in *IOP Conference Series: Materials Science and Engineering*, 2017, doi: 10.1088/1757-899X/263/3/032019.

[48] H. R. Ghaffarian, M. Saiedi, M. A. Sayyadnejad, and M. A. Rashidi, "Synthesis of ZnO nanoparticles by spray pyrolysis method," *Iran. J. Chem. Chem. Eng.*, 2011.

[49] L. Baraket and A. Ghorbel, "Control preparation of aluminium chromium mixed oxides by sol-gel process," *Stud. Surf. Sci. Catal.*, 1998, doi: 10.1016/s0167-2991(98)80233-4.

[50] S. Prasad, V. Kumar, S. Kirubanandam, and A. Barhoum, "Engineered nanomaterials: nanofabrication and surface functionalization," in *Emerging Applications of Nanoparticles and Architecture Nanostructures*, Elsevier, 2018, pp. 305-340.

[51] H. Bagheri Farahani, M. Shahrokhi, and A. Molaei Dehkordi, "Experimental investigation and process intensification of barium sulfate nanoparticles synthesis via a new double coaxial spinning disks reactor," *Chem. Eng. Process. - Process Intensif.*, 2017, doi: 10.1016/j.cep.2017.02.007.

[52] M. Benelmekki and A. Erbe, "Nanostructured thin films–background, preparation and relation

to the technological revolution of the 21st century," in *Frontiers of Nanoscience*, 2019.

[53] A. Kumar and D. Nanda, "Methods and fabrication techniques of superhydrophobic surfaces," in *Superhydrophobic Polymer Coatings*, 2019.

[54] K. Okuyama and W. W. Lenggoro, "Preparation of nanoparticles via spray route," *Chem. Eng. Sci.*, 2003, doi: 10.1016/S0009-2509(02)00578-X.

[55] D. S. Kumar, B. J. Kumar, and H. M. Mahesh, "Quantum nanostructures (QDs): an overview," in *Synthesis of Inorganic Nanomaterials*, Elsevier, 2018, pp. 59-88.

[56] T. M. Abdelghany *et al.*, "Recent advances in green synthesis of silver nanoparticles and their applications: about future directions. A review," *Bionanoscience*, vol. 8, no. 1, pp. 5-16, 2018.

[57] N. I. Hulkoti and T. C. Taranath, "Biosynthesis of nanoparticles using microbes—a review," *Colloids Surfaces B Biointerfaces*, vol. 121, pp. 474-483, 2014.

[58] T. P. Yadav, R. M. Yadav, and D. P. Singh, "Mechanical milling: a top down approach for the synthesis of nanomaterials and nanocomposites," *Nanosci. Nanotechnol.*, vol. 2, no. 3, pp. 22-48, 2012.

[59] S. C. Tjong and H. Chen, "Nanocrystalline materials and coatings," *Mater. Sci. Eng. R Reports*, vol. 45, no. 1-2, pp. 1-88, 2004.

[60] R. J. Varghese, S. Parani, S. Thomas, O. S. Oluwafemi, and J. Wu, "Introduction to nanomaterials: synthesis and applications," in *Nanomaterials for Solar Cell Applications*, Elsevier, 2019, pp. 75-95.

[61] G. Venugopal, M.-H. Jung, M. Suemitsu, and S.-J. Kim, "Fabrication of nanoscale three-dimensional graphite stacked-junctions by focused-ion-beam and observation of anomalous transport characteristics," *Carbon N. Y.*, vol. 49, no. 8, pp. 2766-2772, 2011.

[62] A. H. Hamad, K. S. Khashan, and A. A. Hadi, "Laser ablation in different environments and generation of nanoparticles," *Appl. Laser Ablation Thin Film Depos. Nanomater. Synth. Surf. Modif.*, p. 177, 2016.

[63] G. Mansoureh and V. Parisa, "Synthesis of metal nanoparticles using laser ablation technique," in *Emerging Applications of Nanoparticles and Architecture Nanostructures*, Elsevier, 2018, pp. 575-596.

[64] M. T. Nguyen and T. Yonezawa, "Sputtering onto a liquid: interesting physical preparation method for multi-metallic nanoparticles," *Sci. Technol. Adv. Mater.*, vol. 19, no. 1, pp. 883-898, 2018.